NTP
National Toxicology Program

Report on Carcinogens
Draft Substance Profile for Formaldehyde

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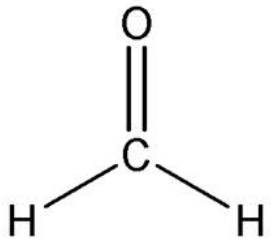
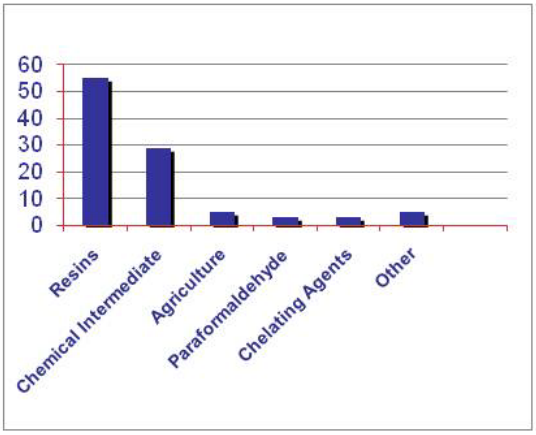
Outline

- Properties and use
- Exposure
- Proposed listing
- Evidence of carcinogenicity
  - Cancer studies in humans
  - Cancer studies in experimental animals
  - Mechanistic studies of carcinogenicity
- Summary

Formaldehyde: Properties and Use

- Simple aldehyde
- Gas at room temperature
- Equilibrium with hydrated form, methylene glycol (methanediol)





USES

Significant U.S. Exposure

- U.S. Production: 6 million tons (2007)
- Occupational Exposure
  - Highest exposures (U.S.): formaldehyde & resin production, plastics and embalming
- Environmental Exposure
  - Ubiquitous
    - Also produced from combustion of organic materials
  - Primary source – indoor air
  - Detected in outdoor air, food, cigarettes and water
- Endogenous Exposure

Proposed Formaldehyde Listing

Formaldehyde is *known to be a human carcinogen*

- Sufficient evidence of carcinogenicity from studies in humans
- Mechanistic studies support the findings in humans

Sufficient Evidence of Carcinogenicity from Studies in Humans

- Consistent findings of increased risks of nasopharyngeal cancer, sinonasal cancer, and myeloid leukemia among individuals with higher exposure
- Cannot be explained by chance, bias, or confounding

Outline: Human Cancer Studies

- Types of human studies
  - Overview
  - Individual studies
- Findings by tumor site
  - Nasopharyngeal cancer
  - Sinonasal cancer
  - Lymphohematopoietic cancers/myeloid leukemia
  - Other cancer sites

Human Cancer Studies: Types of Studies

- Cohort and nested case-control studies of industrial workers
  - Mostly mortality studies
  - Different industries
- Cohort and nested case-control studies of professional groups such as pathologists, funeral directors or embalmers
- Case-control studies: mostly population based
- Numerous meta-analyses

Cohort Studies of Industrial Workers

Cohort Reference	Population	Analyses
NCI Hauptman *et al.* 2003, 2004, Beane Freeman *et al.* 2009	25,619 men & women Companies that use or produce formaldehyde	Internal analysis/quantitative exposure-response analyses: peak, average, cumulative and exposure duration Last recorded exposure was in 1980
NIOSH Pinkerton *et al.* 2004	11,039 men & women Garment workers	SMR analyses by latency, duration of exposure and year of 1st exposure for selected tissue sites
British cohort Coggon *et al.* 2003	14,014 men Chemical workers	SMR analyses for ever exposed, highly exposed, SMR analyses by exposure level (low, medium, high) or duration for stomach and lung cancer
Small cohort studies	100 to < 4,000 Various industries	Ever exposed, 1 incidence study, the rest mortality
Nested case-control studies (mostly lung cancer & lympho-hematopoietic cancers)	Most had small numbers of exposed cases	Mostly evaluated ever exposed

Studies of Professional Groups

- 6 small cohort (mortality) studies
  - Exposure assessed by license or membership in a professional society
- The most informative study: nested case-control study among embalmers from 3 cohort studies: Hauptmann et al. 2009
  - Large study of lymphohematopoietic (LHC) cancers (all LHC = 168, myeloid leukemia = 34)
  - Number of embalmings, duration of working in embalming jobs
  - Quantitative exposure-response analyses: peak, intensity, cumulative exposure

Nasopharyngeal Cancer (NPC): Background

- Rare cancer in many parts of the world (1/100,000)
- Endemic in certain geographical locations, such as southern China
- Histological subtypes
  - Type I keratinizing: differentiated
  - Type II non-keratinizing: differentiated
  - Type III non-keratinizing: non-differentiated

NPC: Informative Studies

- Collective body of case-control studies
  - Multi-center case-control study (Vaughan *et al.* 2000)
    - Largest number of exposed cases
    - Evaluated histological subtypes
    - Evaluated exposure-response relationships
- NCI cohort study
- Other large cohort studies
  - Not informative because of low statistical power, e.g., NIOSH cohort had only 13% power to detect a ≥ 2-fold increase

NPC: Findings

- Consistent findings of increased risk of NPC among individuals with the highest exposure
  - Case-control studies: Olsen *et al.* 1984, Vaughan *et al.* 1986, Roush *et al.* 1987, West *et al.* 1993, Vaughan *et al.* 2000 and Hildesheim *et al.* 2001
  - NCI cohort: SMR = 2.10 (95% CI = 1.05 to 4.21)
- Elevated risks observed after consideration of confounding by tobacco smoking or wood dust or other occupational carcinogens
- Histological subtypes
  - Elevated risk found for differentiated squamous-cell carcinoma and unspecified subtypes: Vaughan *et al.* 2000

NPC: Findings

- Exposure-response relationships
  - Statistically significant (or approaching significance) trends
    - Cumulative exposure: NCI cohort and Vaughan *et al.* 2000
    - Average exposure: NCI cohort
    - Peak exposure: NCI cohort
    - Duration of exposure: Vaughan *et al.* 2000
  - Highest risk estimates found for individuals with:
    - Highest level of exposure: Vaughan *et al.* 1986, 2000, Roush *et al.* 1997, NCI cohort
    - Longest exposure duration: Vaughan *et al.* 1986, 2000, West *et al.* 1993
    - Longest latency: West *et al.* 1993
    - Highest probability of exposure: Vaughan *et al.* 2000

Sinonasal Cancer (SNC): Background

- Comprises cancers of the paranasal sinuses and nasal cavity
- Rare cancer: annual incidence ~1/100,000
- Two major histological types
  - Adenocarcinoma (ADC) - wood dust is a potential confounder
  - Squamous-cell carcinoma (SCC)

SNC: Informative Studies

- Collective body of case-control studies
- Pooled analysis of 12 case-control studies (Luce *et al.* 2002)
  - Greater statistical power: 192 cases ADC, 432 cases SCC
  - Independent exposure analyses
  - Overlap with case-control studies by Hayes *et al.* 1986, Vaughan *et al.* 1986, Luce *et al.* 1993
- Cohort studies
  - Not informative due to low statistical power; for example, only 16% power to detect a ≥ 2-fold increase in the NIOSH cohort
  - Most studies only reported ever exposed

SNC: Findings

- Consistent findings of increased risks for SNC
  - Four case-control studies (Olsen *et al.* 1994, Olsen *et al.* 1986, Hayes *et al.* 1986, Luce *et al.* 1993)
  - Pooled analyses of 12 case-control studies (Luce *et al.* 2002) SMR for high cumulative exposure and ADC
    - Males 3.1 (95% CI = 1.5 to 5.7); 91 exposed cases
    - Females 6.2 (95% CI = 2.0 to 19.5); 5 exposed cases
- Higher risks found among individuals with higher exposure
  - Exposure level (such as cumulative and average): Luce *et al.* 1993, 2002, Hayes *et al.* 1986, Roush *et al.* 1987
  - Earlier start dates: Luce *et al.* 1993
  - Longest duration: Luce *et al.* 1993

SNC: Findings

- Supporting data from cohort studies
  - Statistically significant increase in incidence among male Danish workers exposed to formaldehyde (Hansen and Olsen 1995, 1996)
  - Small non-statistically significant risk in NCI cohort
- Confounding or effect modification: wood dust exposure
  - Increased risks found among subjects with little or no exposure to wood dust or after adjusting for wood dust (Olsen *et al.* 1984, Hayes *et al.* 1986, Hansen and Olsen 1995, 1996)
  - Effect modification (Olsen *et al.* 1984, Luce *et al.* 1993, 2002)
- Histological subtypes: ADC and SCC
  - Increased risks found for both subtypes
  - Some studies suggested stronger risk for ADC

Lymphohematopoietic Cancers (LHC): Background

- Heterogeneous group of tumors
- Classified by tissue distribution at the time of clinical presentation
  - Lymphoma: lymphoid tissue
  - Leukemia: bone marrow and blood
- Also can be classified by stem cell origin: lymphoid vs. myeloid
- Incidence
  - Lymphoma: 22/100,000
  - Leukemia: 12.3/100,000

LHC: Findings

- LHC (combined) and all leukemia combined
  - Excess found in all 6 cohort studies of professional workers and some of the industrial cohorts
  - Positive exposure-response with peak exposure in the NCI cohort
  - Increased risk for non-lymphoid LHC in nested case-control study of embalmers
  - Strongest association for myeloid leukemia

Myeloid Leukemia

- Positive association in the most informative studies
  - Excess risks among individuals with high exposure in 2 of 3 large cohort studies of industrial workers
  - Excess risk in nested case-control study of embalmers
  - Positive exposure-response relationships (NCI and embalmers)
- Unlikely to be explained by confounding
  - Excess risk found in different industries and occupations (e.g., garment workers, formaldehyde production workers and embalmers)
  - NCI study considered exposure to other occupational carcinogens
- Meta-analysis
  - Positive association among workers with the highest exposure RR = 1.90, 95% CI = 1.31 to 2.67 (Zhang *et al.* 2009)

Myeloid Leukemia: Informative Studies

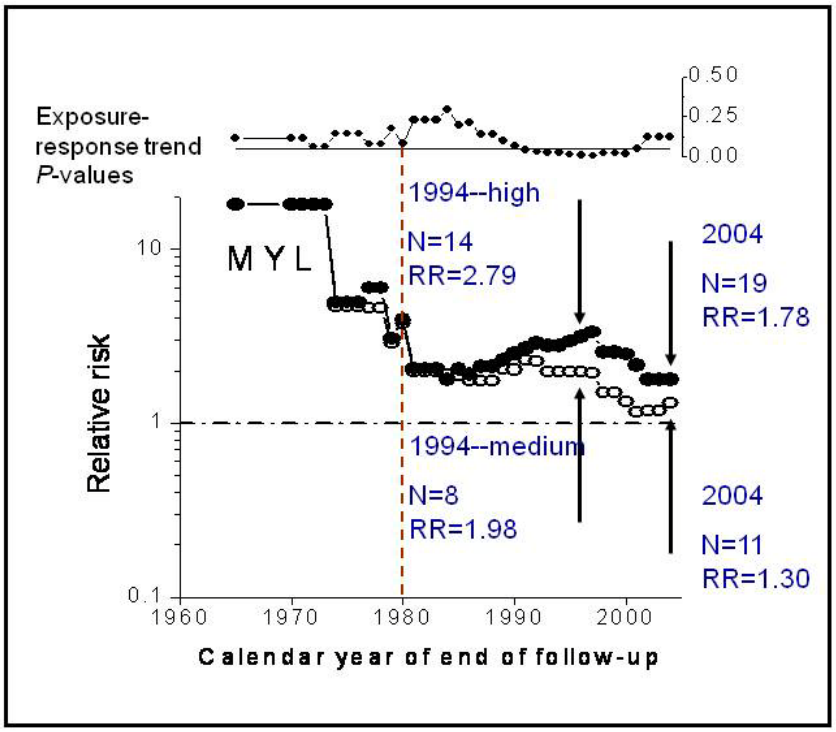
Study	Analyses	Risk Estimate (95% CI); *P* or # cases	Exposure response (*P* Trend)
Nested case-control among embalmers[a] Hauptmann *et al.* 2009	Employment duration Number of embalmings Average exposure Cumulative exposure Peak exposure	OR = 3.9 (1.2–12.5); 0.024 OR = 3.0 (1.0–9.2); 0.057 OR = 2.3 (0.7–7.5); NR OR = 3.1 (1.0–9.6); 0.047 OR = 2.7 (0.9–9.5); NR	***P* = 0.020** *P* = 0.314 ***P* = 0.058** *P* = 0.192 ***P* = 0.036**
NCI industrial workers[b] Beane Freeman *et al.* 2009	Internal analyses Peak exposure	RR = 2.79 (1.08–7.21); 14	*P* = 0.02 (exposed)
NIOSH garment workers Pinkerton *et al.* 2004	1st Exposure < 1963 Employment duration[c] 10 yrs + 10 yrs +/ 20 yrs TSFE	SMR = 1.61 (NR); *P* > 0.05 SMR = 2.24 (1.02–4.25); 9 SMR = 2.55 (1.10–5.03); 8	Highest risks in highest exposure categories
British chemical workers Coggon *et al.* 2003	Myeloid leukemia Ever exposed: leukemia High exposed: leukemia	NR SMR = 0.91 (0.62–1.29); 31 SMR = 0.71 (0.31–1.39); 8	NR

[a] Highest vs. lowest category of exp. in analyses using funeral directors with < 500 embalmings as reference group; OR higher and significant (but unstable) for all these metrics when unexposed funeral directors was the comparison group
[b] Highest category vs. lowest 1994 follow-up (corrected values)
[c] Multiple causes of death analyses
NR = Not Reported; TSFE = Time since first exposure

NCI Cohort and Myeloid Leukemia

- Last known exposure was in 1980
- Median follow-up in the 2004 update was 42 years
- Greatest risks for myeloid leukemia was found for the period of 15 to 25 years since first known exposure
- Magnitude of risk estimate decreased in 2004 follow-up compared to 1994 follow-up
- Similar patterns are seen for other leukemogens
  - increases in risk followed by decreases as the interval after the end of exposure lengthens
- Extending the follow-up time beyond the optimal latency period can result in the addition of non-formaldehyde related leukemia, and thus dilute the effects

Cumulative Risk Estimates for Myeloid Leukemia and Peak Exposure



Beane Freeman *et al.* 2009

Cancer at Other Tissue Sites

- Weaker evidence
  - Head and neck cancer
    - Increases observed in many studies, but no consistent exposure-response relationships
  - Brain
    - Increases observed in all the cohorts of professional workers, but not in the studies of industrial workers
    - No positive exposure-response relationships observed in the nested case-control study of embalmers
- Inconsistent
  - Lung

Summary of Human Studies

- Sufficient evidence of carcinogenicity from studies in humans
  - Consistent findings of increased risks of nasopharyngeal cancer, sinonasal cancer, and myeloid leukemia among individuals with higher measures of exposure to formaldehyde
  - Cannot be explained by chance, bias, or confounding
- Clarifications?

Sufficient Evidence of Carcinogenicity from Studies in Experimental Animals

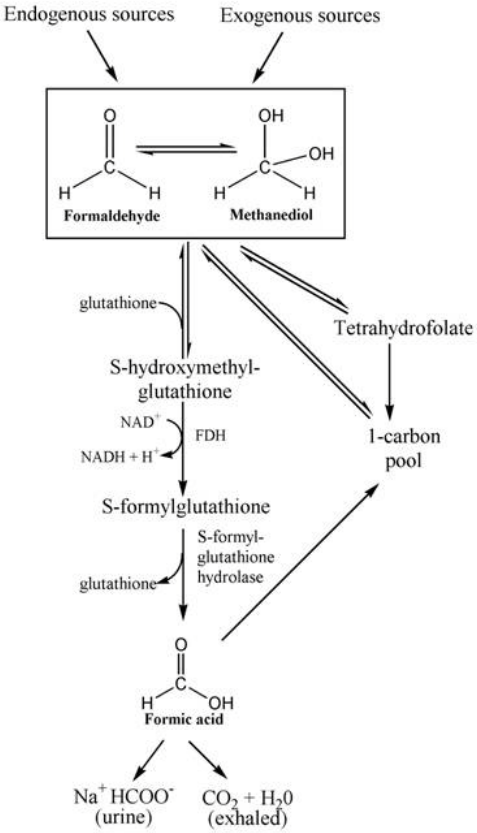
- Formaldehyde causes tumors:
  - In two rodent species: mice and rats
  - By two routes of exposure: inhalation and drinking water
  - At multiple tissue sites: nose, forestomach, muscle (leiomyosarcoma) of intestines and stomach, and testes
- Strongest evidence for nasal tumors
- Promoter of lung, stomach, but not urinary bladder tumors in rats

Nasal Tumors: Inhalation Exposure

- Observed in six studies in rodents including:
  - Male mice
  - Male and female Fischer rats (3 studies)
  - Male Sprague-Dawley rats
  - Male Wistar rats
- Observed after short-term (13 week) exposure (held up to 131 weeks) in rats
- Most tumors were squamous-cell carcinomas, but some nasal carcinoma and polypoid adenomas were also observed
- Squamous-cell carcinomas of the nasal cavity are very rare in mice and rats
  - In NTP studies, no tumors have been reported in more than 2,800 historical control mice, and more than 1,300 historical control rats

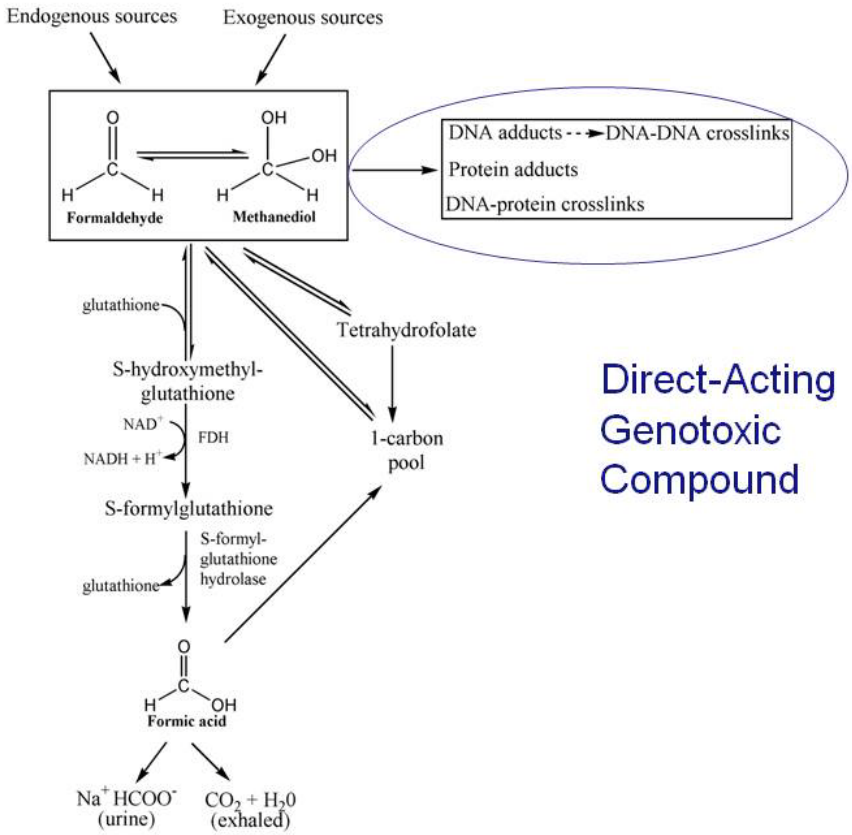
Outline: Mechanistic Evidence

- Metabolism
- Multiple modes of actions
- Mechanistic data: nasal tumors
- Mechanistic data: leukemia
  - Background
  - Plausibility of inducing cancer or toxic effects at distal sites
  - Other proposed mechanisms of formaldehyde-induced leukemogenesis
  - Hematological toxicity and summary



Metabolism

- Rapidly metabolized
  - Half-life in plasma in monkeys ~1 to 1.5 min
- FDH (ADH5) ubiquitously expressed in all human tissues from embryos through adults
- Metabolism pathway in all species



Direct-Acting Genotoxic Compound

Mechanistic Considerations: Multiple Modes of Action

- Direct-acting genotoxic compound
  - Positive in almost all *in vitro* systems
  - Also causes gene mutation, chromosome breakage and aneuploidy
- Other potential modes of action
  - Glutathione depletion
  - Epigenetic effects
  - Oxidative stress
  - Cytotoxicity-induced cellular proliferation

Mechanistic Considerations: Nasal Tumors

- Inhalation exposure causes genetic damage in the nasal tissue in both experimental animals and humans
  - DNA-protein cross links correlate with tumor incidence, severity and anatomic location of nasal lesions in laboratory animals
  - Increased micronuclei frequency found in nasal epithelium of formaldehyde exposed workers
  - DNA cross links related to chromosomal effects (SCE and MN) in V79 cells
  - p53 mutations found in formaldehyde-induced cancers in rats
- Airway deposition
  - Regional formaldehyde flux correlates with anatomical distribution of formaldehyde-induced lesions in rats
- Cytotoxicity-induced cellular proliferation
  - Cellular proliferation correlates with tumor incidence in rats

Leukemia: Background

- Lymphohematopoietic cancers (LHC)
  - Blood cells arise from a common stem cell, which forms two major progenitor cells: myeloid and lymphoid stem cells
  - Different types of LHC arise from damaged stem cells at different levels of hematopoietic and lymphoid development
- Leukemia
  - Most agents that cause leukemia do so by directly damaging stem cells in the bone marrow, which give rise to leukemic stem cells
  - Formaldehyde can damage chromosomes, but can it reach the target cells of interest, that is early blood cells and progenitor cells?

Plausibility of Formaldehyde-Induced Leukemia

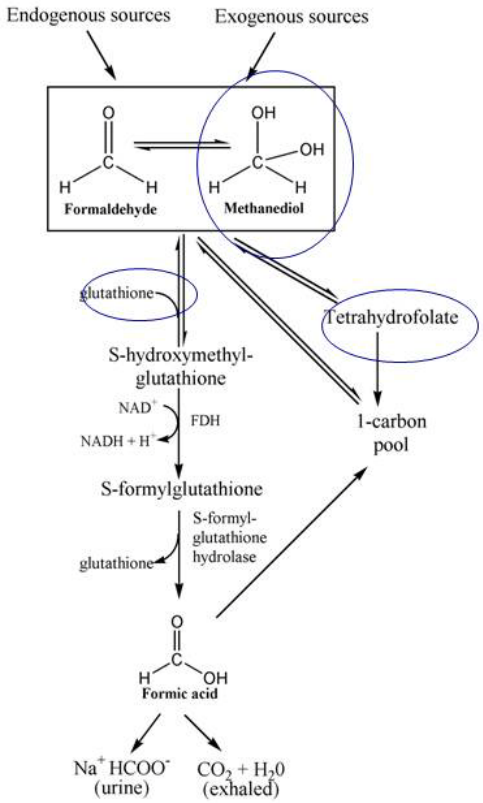
- Can formaldehyde reach the bone marrow, or cause toxicity, genotoxicity or cancer at distal sites?
  - Formaldehyde is highly reactive
  - No increase in blood levels of formaldehyde after exogenous exposure in rats, monkeys, and humans (Heck and colleagues)
  - DNA adducts or DNA protein cross links have not been detected at distal sites in experimental animals (Lu *et al.* 2010)
  - Many studies on cytotoxicity endpoints in animals have been negative
- However, toxic effects have been observed at distal sites after inhalation exposure in humans and experimental animals

Toxic Effects at Distal Sites

- Humans (Inhalation)
  - Serum albumin adducts in formaldehyde exposed laboratory workers
  - Hematological toxicity
  - Genotoxicity: DNA-protein cross links, DNA strand breaks, micronuclei in peripheral blood lymphocytes, some evidence for chromosomal aberrations
- Experimental animals (Inhalation)
  - Toxicity at distal sites: liver, nervous system, testes, spleen
  - Genotoxicity: DNA strand breaks in liver and lymphocytes, dominant lethal mutations in rats, heritable mutations in mice
- Bone marrow: experimental animals
  - CA in rats after inhalation exposure (conflicting findings)
  - CA and aneuploidy in mice after oral exposure

Possible Mechanisms for Distribution of Formaldehyde

- Equilibrium with methanediol
- Binding to reversible products such as glutathione, amino acids and folic acid



Leukemia: Other Proposed Mechanisms

Formaldehyde could cause leukemia by mechanisms that do not require it to reach the bone marrow (Zhang *et al.* 2009)

- Damage to circulating stem cells in the blood
  - Stem cells have been identified in peripheral circulation and can circulate back into the bone marrow
  - Cytogenetic damage in lymphocytes of formaldehyde-exposed workers
- Damage to stem cells in the nasal turbinates or olfactory mucosa
  - Micronuclei induction in nasal tissues of formaldehyde-exposed workers
  - Stem cells found in olfactory epithelial cells from nasal passage
    - Repopulate hematopoietic tissue of irradiated rats to form progenitor cells of multiple lineages

Leukemia: Hematological Toxicity & Summary

- Adverse hematological effects would be expected if there is damage to hematopoietic stem or progenitor cells
  - Review of Chinese literature: some evidence
    - Decreased WBC, platelets & hemoglobin
  - Biomonitoring study of Chinese workers exposed to formaldehyde
    - Decreased WBC, granulocytes, platelets, RBC, lymphocytes
    - Higher frequency of aneuploidy of chromosomes 7 & 8 in myeloid progenitor cells
  - Formaldehyde exposure caused a decrease in colony forming progenitor cells (BFU-E, CFU-E, CRU-GEMM) *in vitro*
- Mechanisms not known
  - Available evidence does not indicate that such mechanisms are implausible

Summary: Preliminary Listing Recommendation

List formaldehyde as *known to be a human carcinogen*

- Sufficient evidence of carcinogenicity from studies in humans demonstrating a causal relationship with nasopharyngeal cancer, sinonasal cancer and myeloid leukemia
- Mechanistic studies support the finding in humans
  - Formaldehyde is a direct-acting genotoxin
  - Formaldehyde causes key events that are associated with carcinogenicity
- Formaldehyde causes cancer in experimental animals
  - Site concordance: nasal cancer